EVALUATION OF POSSIBLE INTERACTION AMONG DRUGS CONTEMPLATED FOR USE DURING MANNED SPACE FLIGHTS

PART I: SUMMARY FROM PROGRESS REPORT DATED OCTOBER 31, 1973

PART II: PROGRESS REPORT FOR THE PERIOD NOVEMBER 1973 TO JUNE 1974

FINAL REPORT
for the period
JULY 1972 TO JUNE 1974

prepared for

MANNED SPACECRAFT CENTER
NATIONAL AERONAUTICS
AND SPACE ADMINISTRATION
HOUSTON, TEXAS
CONTRACT NAS-9-12970

by

ARTHUR D. LITTLE, INC.
CAMBRIDGE, MASSACHUSETTS 02140

C-74804

JULY 31, 1974

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FOREWORD

This program was undertaken to evaluate possible interactions among drugs contemplated for use during manned space flights. It was carried out during the period July 1, 1972 to June 30, 1974 and was the subject of our proposals dated March 10, 1972 and May 14, 1973, in response to RFP No. 9-13B32-79-2-185P and RFP No. 9-13B631-4-3177P.

A Progress Report, dated October 31, 1973, was issued covering the work completed during the period July 1972 to September 1973. This final report includes the summary from the October 1973 report and the details of the work from November 1973 to June 1974 when the program was terminated.

Dr. Vernon Carter, formerly of the NASA Manned Spacecraft Center, was the original Technical Officer for that agency. He has recently been replaced by Dr. Harold Kaplan. Dr. David W. Yesair, Dr. Francis J. Bullock (former Arthur D. Little, Inc. staff member), Dr. Philip S. Thayer, Ms. Marianne Callahan, and Mr. James Braun comprised the Arthur D. Little, Inc. project team.

PART I: SUMMARY FROM PROGRESS REPORT FOR THE PERIOD JULY 1972 TO SEPTEMBER 1973.

Possible interactions among drugs contemplated for use during manned spaceflights have been studied in several animal species. The following seven drugs were investigated: nitrofurantoin, chloral hydrate, hexobarbital, phenobarbital, flurazepam, diphenoxylate, and phenazopyridine. Particular combinations included: chloral hydrate, hexobarbital or flurazepam with nitrofurantoin; phenobarbital or flurazepam with phenazopyridine; and diphenoxylate with two dose formulations of nitrofurantoin.

Specifically, rat liver microsomes were used to determine whether chronic dosage with nitrofurantoin would inhibit rates of oxidative drug metabolism. Rates of hexobarbital oxidation, N-demethylation of meperidine, and reduction of *p*-nitrobenzoic acid were studied in control and nitrofurantoin-treated rats.

Pharmacological studies were carried out to determine whether premedication of rats with nitrofurantoin would result in potentiation of activity of hexobarbital, chloral hydrate, or flurazepam. This was determined for chloral hydrate and hexobarbital by studies of sleeping times determined by loss of the righting reflex. Flurazepam was investigated by use of the inclined screen test. No potentiation of activity was found between control and nitrofurantoin-treated animals.

Studies were carried out in several species to determine whether induction of liver microsomal enzymes would increase the tendency of phenazopyridine to produce methemoglobin *in vivo*. Animals were premedicated with phenobarbital, a known inducer of azoreductase, and in a separate experiment with flurazepam, before administration of phenazopyridine. Methemoglobin production was determined in each animal after receiving phenazopyridine. No evidence was found for increased production of methemoglobin in the rat, dog, or rabbit that could be attributed to increased amounts of microsomal enzymes.

The final group of experiments was conducted to determine whether premedication of the dog with diphenoxylate would result in altered bioavailability of Furadantin® or Macrodantin®. The rate of drug excretion in the urine was determined and a plot of percent drug absorbed versus time was constructed for each dosage form, with and without diphenoxylate pretreatment. Drug in the urine was determined by both chemical and microbiological methods. Two doses of diphenoxylate were studied in four female beagles with each dog serving as its own pre-diphenoxylate control. Diphenoxylate was found to alter substantially the excretion pattern of both forms of nitrofurantoin, generally increasing total absorption.

On the basis of this work, the present contract was extended to include nitrofurantoin/diphenoxylate studies in man. The clinical study was carried out by Medical and Technical Research Associates, Inc., Needham, Massachusetts. Six subjects received both dosage forms of nitrofurantoin alone, and in combination with diphenoxylate. The results of these studies are inconclusive. The mechanism of action and an explanation of the interaction between diphenoxylate and nitrofurantoin still remains unclear. In man, the interaction does not appear to be significant, affecting only two subjects out of six and with only one dose formulation (Furadantin®).

PART II: PROGRESS REPORT FOR THE PERIOD OCTOBER 1973 TO JUNE 1974.

SUMMARY

An extensive investigation has been carried out looking into the pharmacodynamics of diphenoxylate interaction with certain drugs; specifically, pentobarbital, phenobarbital, secobarbital, hexobarbital, chloral hydrate, flurazepam, and meperidine. The various barbiturates were chosen because they differ widely in their rates of absorption, metabolism, and excretion, and in their duration of pharmacological activity. Flurazepam was included because it, along with chloral hydrate and secobarbital, is carried in the Sky Lab medical kit. Meperidine was investigated for it appeared that such a combination might yield additional information concerning the interaction between diphenoxylate and CNS depressant drugs.

Pharmacological studies were carried out to determine whether premedication of rats with diphenoxylate would result in potentiation of activity with the second drug. Chloral hydrate, flurazepam, and the barbiturates were investigated by measuring sleeping times in control and diphenoxylate-treated rats. Pretreatment with diphenoxylate was found to potentiate the activity of all the above-named sedative drugs. When the concentration of the sedative drug was decreased, diphenoxylate still increased the sedative-induced sleeping times. Diphenoxylate-dosed rats given half the original dose of either of two barbiturates or chloral hydrate, had sleeping times comparable to control animals which received the full dose of the sedative.

Meperidine analgesia was measured by the Eddy hot plate technique. Initial analgesic effect in diphenoxylate-pretreated rats was greater than in control animals and, although the peak of analgesic activity (measured at 20 min following meperidine injection) was the same in both groups, the level of activity subsequently remained higher in the diphenoxylate-pretreated rats.

More definitive pharmacological studies were carried out with

secobarbital and chloral hydrate in an attempt to delineate the mechanism of interaction between diphenoxylate and sedative drugs. Experiments were conducted in which the concentration of diphenoxylate and its time of administration, with respect to secobarbital and chloral hydrate, were varied. As the dose of diphenoxylate was increased, the duration of sleeping time with either drug also increased. With secobarbital, one also saw an increase in sleeping times as the length of time between dosing with diphenoxylate and dosing with secobarbital increased. With chloral hydrate as the sedative drug, the only significant increase in sleeping times occurred with the highest dose of diphenoxylate. Also, as the time increased between dosing with diphenoxylate and dosing with chloral hydrate at this high dose, the sleeping times, though still significantly greater than in control rats, tended to decrease.

Physiological disposition studies were carried out to evaluate the possibility that the pharmacological effect might relate closely to the pharmacokinetics of the sedative drug in diphenoxylate-pretreated rats. Radioactive sedative drugs were administered intravenously to both normal and diphenoxylate-pretreated rats. Drug equivalents in plasma, urine, and feces were determined. Plasma curves for both groups of animals were statistically equivalent, as was urinary excretion of drug equivalents. Fecal output in diphenoxylate-pretreated rats is reduced; therefore, total excretion of drug equivalents in diphenoxylate-pretreated animals is lower than in control animals after 48 hr.

The concentration of a given sedative drug in brain is relatively constant upon waking, regardless of the duration of sleep. Thus, we evaluated the effect of diphenoxylate on the concentration of secobarbital and flurazepam equivalents in brain and blood. Total radioactivity was measured in the blood and brain of control and diphenoxylate-pretreated animals at various time periods following the administration of C^{14}-secobarbital or C^{14}-flurazepam. The concentration of drug equivalents in the blood and brain was essentially the same for both control and diphenoxylate-pretreated animals with both drugs.

Although sleeping times are approximately double in diphenoxylate animals, the concentration of drug equivalents in both the brain and blood of these animals at waking is less than that of controls. One would have expected equivalent concentrations of secobarbital and flurazepam if sedative drugs mediated the increase in sleeping times.

The increase in pharmacological activity when sedative drugs are used in combination with diphenoxylate cannot be explained on the basis of inhibition of drug metabolism. Studies *in vitro* performed under our first NASA contract, in which microsomal hexobarbital oxidase activity was measured in control and diphenoxylate-treated animals, showed no difference between the two groups as defined by their K_m and V_{max} values. Also, the physiological disposition studies just completed found no pharmacokinetic differences of sedative drugs in diphenoxylate-treated animals. Most likely, when sedative drugs are administered, the diphenoxylate *per se* and/or metabolites which remain in the brain for such long periods of time produce an additive effect that is manifested in increased sleeping times.

I. INTRODUCTION

The general approach to the use of drugs by astronauts during space flights has been cautious, but in-flight medical problems requiring their use have occurred. With the advent of the Sky Lab program, the probability that astronauts may find it necessary to take one or more of the drugs carried in their medical kits greatly increased. As has been reviewed elsewhere (1), results of epidemiological studies of adverse drug effects were of great consequence in bringing attention to drug interactions. Of particular importance, a direct relationship between the number of drugs used and the probability of adverse drug reactions clearly emerged from these studies. Unquestionably, use of drugs in combination carries with it an increased probability of an adverse drug effect.

Adequate clinical data on risk/benefit ratios for particular drugs are just now beginning to emerge. Similar data for drugs used in combination are generally unavailable. The relative probabilities for therapeutic mishap are highest for antimicrobials, cardiac drugs, and hypnotics and sedatives. These classes are all heavily represented in the list of drugs now contemplated for possible use during manned space flight. With the Sky Lab program involving longer residence for astronauts in space, this project was concerned with the increased possibility that drugs would be taken in combination, with unknown effects.

II. BACKGROUND

In our previous programs for NASA, we have studied the effect of spacecraft environment on drug metabolism and on the pharmacological activity of drugs. We have just finished a program designed to investigate the potential interaction for various drug combinations. Generally, we have concluded that most drugs under spacecraft environment or in combination had no significant adverse effects. Diphenoxylate (I), however, did appear to alter the pharmacodynamics of at least three classes of drugs.

In our first NASA-sponsored studies, diphenoxylate potentiated secobarbital and, to a lesser extent, hexobarbital-induced sleeping times in rats. Diphenoxylate had been given 16-18 hr prior to the barbiturates. Since barbiturate metabolism by liver microsomes *in vitro* was unchanged in diphenoxylate-treated rats compared to control animals, the potentiation does not appear to involve inhibition of barbiturate metabolism by diphenoxylate.

In our second NASA program, we have recently shown that diphenoxylate apparently increased the overall absorption of nitrofurantoin, given either as the macrocrystalline dosage form or the microcrystalline dosage form to dogs. In these studies, as before, diphenoxylate was given to the dog 16-18 hr prior to the oral administration of nitrofurantoin.

The striking feature of the drug interaction between diphenoxylate and barbiturates in rats, and between diphenoxylate and nitrofurantoin in dogs was the length of time between dosing the two drugs. Diphenoxylate has been shown to be extensively metabolized in rats, dogs, and man (2,3). In bile-cannulated rats, 90% of the administered label was recovered in the bile (3). The major excretory pathway is the feces and the rate of fecal excretion of the metabolites is slow. Thus, the extensive

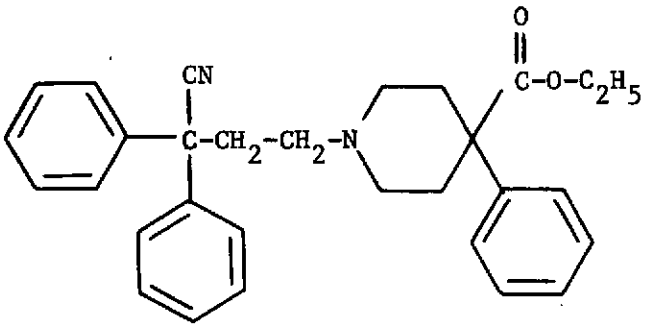
biliary secretion and slow fecal excretion is consistent with having an enterohepatic cycle for diphenoxylate and its metabolites. A metabolite of diphenoxylate, difenoxine, (II)(R15403), has been shown to be responsible for the pharmacological activity associated with diphenoxylate (4-7) and the pharmacokinetics of difenoxine in man and animals (4-7) are similar. If diphenoxylate enters an enterohepatic cycle, then one would expect a long biological half-life of the active component. Thus, any interaction between diphenoxylate and a second drug many hours after the diphenoxylate treatment could reasonably be expected.

The mechanism of interaction between diphenoxylate and nitrofurantoin is probably due to the enhanced rate of absorption from the G.I. tract. For example, nitrofurantoin is highly water insoluble and the particle size is extremely important in its rate of solubilization by the intestinal fluids. Since diphenoxylate decreases the G.I. motility, the increased absorption of both the macro- and microcrystalline nitrofurantoin probably is due to increased residence time of the drugs in the G.I. tract.

The purpose of extending the present contract was to help delineate the mechanism of interaction between diphenoxylate and certain sedative drugs. Diphenoxylate is a meperidine congener that is used exclusively in the treatment of diarrhea. Man in space takes diphenoxylate as needed to control gastrointestinal motility. Other drugs, such as sedatives and stimulants, may be used occasionally. The combination of barbiturates and diphenoxylate has been used by United States astronauts on prolonged space flights. The manufacturer's literature on diphenoxylate states that the drug should be used with caution, if at all, in individuals simultaneously receiving addicting drugs (tranquilizers, alcohol), or barbiturates, because of potentiating effects (8). No data on interactions between barbiturates and diphenoxylate appear in the open literature and the manufacturer has informed us that no pharmacological work on the combination has been done.

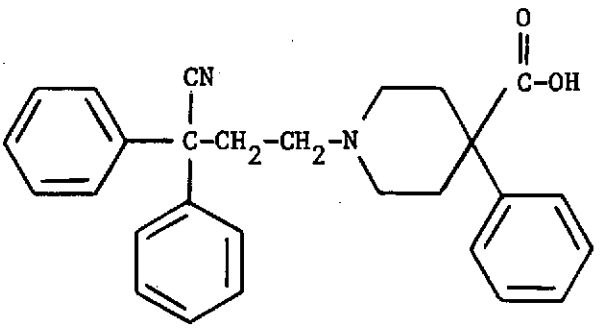
We have thus proceeded to evaluate the effect of diphenoxylate on the drug-induced sleeping time and pharmacodynamics of several barbiturates, chloral hydrate, flurazepam, and meperidine.

DIPHENOXYLATE



I

DIFENOXINE



II

III. MATERIALS AND METHODS

Pharmacological studies were set up to measure the effect of diphenoxylate on sedative-induced sleeping times. Sleeping times were recorded as the interval between loss and return of the righting reflex. The criteria for measuring the loss and return of the reflex was the inability and ability, respectively, of the animal to right itself within 30 sec when placed on its back (9).

Diphenoxylate at 10 mg/kg was given by intubation 16-18 hr prior to administering the sedative drug intravenously. Control animals received comparable doses of the vehicle (1% Tween 80/saline) at that time. The dose for the sedative drug was based on the amount necessary to produce a convenient, measurable sleeping time of 30-45 min in normal rats.

Separate experiments were carried out using the following drugs: pentobarbital, phenobarbital, secobarbital, hexobarbital, chloral hydrate, and flurazepam. Ten control rats and ten diphenoxylate-pretreated rats were used in each experiment. Means and standard deviations were calculated for each group and the "t" test for significance run with the results. Significance was defined as test results that differ from control values by a "p" value of less than 0.05, as measured by the "t" test. In other words, the probability (p) that the values obtained with treated animals and values obtained with control animals are different by chance alone is less than 5%.

An additional group of sleeping time experiments were run with pentobarbital, secobarbital, chloral hydrate, and flurazepam, measuring the effect of decreasing the dose of the sedative drug while all the other factors were kept constant. Diphenoxylate at 10 mg/kg was again administered by intubation 16-18 hr prior to the sedative drug while control animals received 1% Tween 80/saline. The sedative drug was then administered intravenously at the full dose and at one-half the original

dose to groups of control and diphenoxylate-pretreated rats. Sleeping times were measured and the results were analyzed statistically as described above.

A final group of sleeping time experiments was run with secobarbital and chloral hydrate. This time, the dose of the sedative drug remained constant while the dose of diphenoxylate and the time between the administration of diphenoxylate and the sedative drug were varied. Diphenoxylate was given at doses of 0.16, 0.625, 2.5, and 10 mg/kg, and at 1, 8, 16, and 40 hr before dosing with the sedative drug. Again, sleeping times and results were calculated as above.

Meperidine-induced analgesia was evaluated by use of the Eddy hot plate technique (10). With a hot plate maintained at 58°C, analgesic activity was measured over a period of 2 hr, using a 20 sec cut-off time (defined as 100% response). Percent analgesia was determined in control and diphenoxylate-pretreated rats (20 animals/group) from the area under the curve found by plotting the response time observed at various time periods after the administration of meperidine.

Experiments were set up to measure the effect of diphenoxylate on the plasma pharmacokinetics of the sedative drugs. Radioactive drug was administered intravenously, at a dose level comparable to that used in the pharmacological experiments, to both control and diphenoxylate-pretreated rats (10 mg/kg p.o., 16-18 hr previously). Blood was obtained from the optic vein at 1, 2, 5, 10, 15, 30, 45, 60, 75, 90 min and 2, 3, 4, 6, 8, 12, and 24 hr. Radioactivity in plasma was determined directly by liquid scintillation counting (11).

Urine and feces were collected from an additional group of normal and diphenoxylate-pretreated rats dosed as above with radioactive drug. Coprophagy was prevented by the tail-cup technique (12) and these animals were housed in individual No. 110 metabolism cages (Maryland Plastics, Inc., New York, N.Y.) for collection of urine and feces.

Collection periods were 0-3, 3-6, 6-12, 12-24, and 24-48 hr after the administration of the radioactive drugs. Radioactivity in urine was determined directly by liquid scintillation counting, and in feces after combustion by the Peterson technique (13).

The final group of experiments was designated to evaluate the concentration of secobarbital and flurazepam equivalents in blood and brain of control and diphenoxylate-pretreated rats. Diphenoxylate was administered orally as before at 10 mg/kg, 16-18 hr prior to all animals receiving C^{14}-secobarbital i.v. at 30 mg/kg or, in a separate experiment, C^{14}-flurazepam i.v. at 60 mg/kg. Four control and four diphenoxylate-pretreated rats were sacrificed at 2, 5, 10, 20, 45, 60, 90, and 120 min after administration of the radioactive drug. In addition, 6 control and 6 diphenoxylate-pretreated rats were sacrificed immediately upon waking. Total equivalents were determined by direct counting of the plasma and following combustion of the brain samples.

IV. RESULTS AND DISCUSSION

A. PHARMACOLOGICAL STUDIES

1. Effect of Diphenoxylate on Sedative-Induced Sleeping Times. Preliminary dose-ranging with the sedative drugs, using male, Sprague-Dawley rats, established that the following doses produced sleeping times of convenient length for control animals: pentobarbital, 20 mg/kg i.v., phenobarbital, 110 mg/kg i.v., secobarbital, 30 mg/kg i.v., hexobarbital, 80 mg/kg i.v., chloral hydrate, 200 mg/kg i.v., and flurazepam, 60 mg/kg i.v.

The selection of the initial dose of diphenoxylate was based on the results of Janssen *et al.* (14). Janssen studied the influence of an oral dose of diphenoxylate (R1132) on fecal excretion by Wistar rats by quantitation of the number of fecal pellets passed over a period of 24 hr. At 10 mg/kg, significant effects were produced which persisted for at least 48 hr after a single oral dose. At 1 mg/kg, a smaller but measurable effect was seen on the first day following dosage but not on the second day. Diphenoxylate was devoid of significant analgesic activity in mice and rats following subcutaneous doses of up to 80 mg/kg of body weight. To avoid the tedious and time comsuming task of dose-ranging with diphenoxylate, we elected to use the initial oral dose of 10 mg/kg.

The effect of diphenoxylate on sleeping times with the "Skylab" sedatives, secobarbital, chloral hydrate, and flurazepam and three additional barbiturates are summarized in Table 1 and presented in Tables 2-8. In all cases, the sleeping times for treated animals were approximately twice those for control animals.

In an experiment reported to NASA (Contract NAS 9-9806) in October 1970, chloral hydrate, administered i.p. at 200 mg/kg, showed no potentiation of sleeping times in diphenoxylate-treated rats compared to a control group. However, based on our present extensive work with diphenoxylate and chloral hydrate (Tables 3 and 4), we will have to

TABLE 1

SUMMARY: EFFECT OF DIPHENOXYLATE ON DRUG-INDUCED SLEEPING TIMES IN RATS

		Sleeping Times - min	
Drug	Dose (mg/kg i.v.)	Control	Diphenoxylate
Secobarbital	30	46	94
Chloral hydrate	200	36	74
Flurazepam	60	23	46
Pentobarbital	20	29	66
Phenobarbital	110	53	97
Hexobarbital	80	28	46

TABLE 2

EFFECT OF DIPHENOXYLATE ON SECOBARBITAL-INDUCED[a] SLEEPING TIMES IN RATS

Control Sleep Times (min)	Diphenoxylate-dosed[b] Sleep Times (min)
41	91
39	105
41	65
34	93
47	73
54	110
52	119
56	98
Mean ± S.D. 45.5 ± 7.9	94.3 ± 18.2

"t" 6.96

p >0.01

[a] 30 mg/kg i.v.

[b] 10 mg/kg p.o., 17 hours prior to dosing with secobarbital

TABLE 3

EFFECT OF DIPHENOXYLATE ON CHLORAL HYDRATE-INDUCED[a] SLEEPING TIMES IN RATS

Experiment 1

Control Sleep Times (min)	Diphenoxylate-dosed[b] Sleep Times (min)
40	69
37	67
39	71
41	77
30	71
40	71
38	83
24	81
28	76
37	72
35	73
37	74
35	78
32	73
40	
37	
36	
38	
Mean ±S.D. 35.78±4.6	Mean ±S.D. 74.00±4.5

"t" 23.54

p >0.01

a 200 mg/kg i.v.

b 10 mg/kg p.o., 17 hr prior to dosing with chloral hydrate

TABLE 4

EFFECT OF DIPHENOXYLATE ON CHLORAL HYDRATE-INDUCED[a] SLEEPING TIMES IN RATS.

Experiment 2

Control Sleep Times (min)	Diphenoxylate-dosed[b] Sleep Times (min)
8	18
8	13
6	10
7	57
6	56
2	46
5	22
4	21
14	60
16	51
13	29
13	58
18	30
11	58
4	53
9	51
8	66
7	49
7	42
15	17
14	18
9	48
5	14
10	52
14	43
	12
	50
Mean ±S.D. 9.32±4.3	Mean ±S.D. 38.7±18.1

"t" 7.91

p >0.01

[a] 200 mg/kg i.p.

[b] 10 mg/kg p.o., 17 hr prior to dosing with chloral hydrate.

TABLE 5

Effect of Diphenoxylate on Flurazepam-Induced[a] Sleeping Times in Rats

Control Sleep Times (min)	Diphenoxylate-dosed[b] Sleep Times (min)
25	54
26	46
16	49
32	56
30	49
25	39
19	40
21	34
18	41
23	56
23	37
11	54
24	39
Mean ±S.D. 22.54±5.6	45.69±7.8

"t" 8.69

p >0.01

[a] 60 mg/kg i.v.

[b] 10 mg/kg p.o., 17 hr prior to dosing with flurazepam.

TABLE 6

EFFECT OF DIPHENOXYLATE ON PENTOBARBITAL-INDUCED[a] SLEEPING TIMES IN RATS

Control Sleep Time (min)	Diphenoxylate-dosed Sleep Time[b] (min)
34	59
35	75
26	75
19	70
37	54
25	63
28	69
26	
Mean ±S.D. 28.7 ± 6.1	66.4 ± 8.0

"t" 10.3

p >0.01

[a] 20 mg/kg i.v.

[b] 10 mg/kg p.o. 17 hr prior to dosing with pentobarbital.

TABLE 7

EFFECT OF DIPHENOXYLATE ON PHENOBARBITAL-INDUCED[a] SLEEPING TIMES IN RATS

Control sleep times (min)	Diphenoxylate-dosed[b] sleep times (min)
56	91
54	99
56	100
58	98
52	102
39	94
62	94
52	95
48	101
	94
Mean ± S.D. 53.0±6.6	96.8±3.7

"t" 18.10

p >0.01

[a] 110 mg/kg i.v.

[b] 10 mg/kg p.o., 17 hr prior to dosing with phenobarbital

TABLE 8

EFFECT OF DIPHENOXYLATE ON HEXOBARBITAL-INDUCED[a] SLEEPING TIMES IN RATS

Control Sleep Times (min)	Diphenoxylate-dosed[b] Sleep Times (min)
32	51
25	39
30	58
32	41
28	54
25	40
27	41
31	49
32	41
22	44
Mean ± S.D. 28.4 ± 3.6	45.8 ± 6.7

"t" 7.23

p >0.01

[a] 80 mg/kg i.v.

[b] 10 mg/kg p.o., 17 hours prior to dosing with hexobarbital

reverse the original conclusion and now state that there is a definite potentiation effect when diphenoxylate and chloral hydrate are used in combination.

Tables 9-13 demonstrate the effect of administering the sedative drug at two dose levels, keeping the diphenoxylate dose constant at 10 mg/kg. As seen in summary Table 9, diphenoxylate increased the sedative-induced sleeping times for four sedatives given at half the dose. What is of note, however, is that diphenoxylate-dosed rats given half the original dose of two barbiturates (Tables 10 and 11) and chloral hydrate (Table 12) had sleeping times somewhat less than control animals which received the full dose. Reducing the dose of flurazepam to diphenoxylate-pretreated rats resulted in sleeping times generally greater than control animals which received the full dose. Also significant is the finding that the lower dose of sedative drug may not produce a sleeping time, whereas the same dose to diphenoxylate-treated animals always produces sedation (Table 13).

2. Effect of Diphenoxylate on Meperidine-Induced Analgesia.

In our first NASA contract (NAS 9-9806) we studied the effect of spacecraft environment on the metabolism of meperidine *in vitro* and on meperidine-induced analgesia in the rat. We found that the 5 psia 100 percent oxygen environment was not sufficiently stressful to alter the action of meperidine or its rate of biotransformation. This expertise was used in the present study to investigate the interaction between diphenoxylate and meperidine. The Eddy hot plate technique was used to measure meperidine-induced analgesia and the control and diphenoxylate-pretreated groups were compared in terms of "percent analgesia" observed over a 2-hr test period. Percent analgesia is determined from the area under a curve formed by plotting the response times observed at various time periods.

The mean response times observed in control and diphenoxylate-pretreated rats are shown in Figures 1 and 2. There were 20 animals per

TABLE 9

SUMMARY: EFFECT OF DIPHENOXYLATE ON DECREASED DOSES OF SEDATIVE DRUGS

Drug	Dose (mg/kg)	Sleeping Time - min	
		Control	Diphenoxylate
Secobarbital	30	46	78
	15	15	36
Chloral hydrate	200	41	59
	100	0	32
Flurazepam	60	21	43
	30	8	32
Pentobarbital	20	38	55
	10	5	22

TABLE 10

EFFECT OF DIPHENOXYLATE ON SECOBARBITAL-INDUCED SLEEPING TIMES IN RATS

Secobarbital 30 mg/kg i.v.		Secobarbital 15 mg/kg i.v.	
Control Sleep Times (min)	Diphenoxylate-dosed[a] Sleep Times (min)	Control Sleep Times (min)	Diphenoxylate-dosed[a] Sleep Times (min)
37	85	10	39
53	75	10	33
48	79	16	30
43	73	17	40
52	80	17	27
41	72	18	45
47	67	14	44
49	84	14	37
49	71	14	37
45	90	16	25
Mean ±S.D.	Mean ±S.D.	Mean ±S.D.	Mean ±S.D.
46.4±4.97	77.6±7.2	14.6±2.8	35.7±6.8
"t" 11.3		"t" 9.1	
p >0.01		p >0.01	

[a] 10 mg/kg p.o., 17-18 hr prior to dosing with secobarbital

TABLE 11

EFFECT OF DIPHENOXYLATE ON PENTOBARBITAL-INDUCED SLEEPING TIMES IN RATS

Pentobarbital 20 mg/kg i.v.		Pentobarbital 10 mg/kg i.v.	
Control Sleep Times (min)	Diphenoxylate-dosed[a] Sleep Times (min)	Control Sleep Times (min)	Diphenoxylate-dosed[a] Sleep Times (min)
46	48	12	25
48	57	2	15
47	58	12	17
29	57	9	18
29	49	4	28
39	63	4	17
35	55	3	27
38	51	-[b]	27
32	67	-[b]	23
35	44	-[b]	25
Mean ±S.D.	Mean ±S.D.	Mean ±S.D.	Mean ±S.D.
37.8±7.2	54.9±7.1	4.60±4.7	22.2±4.95
"t" 5.38		"t" 8.28	
p >0.01		p >0.01	

[a] 10 mg/kg p.o. 17-18 hr prior to dosing with pentobarbital

[b] Animal did not lose righting reflex

TABLE 12

EFFECT OF DIPHENOXYLATE ON CHLORAL HYDRATE-INDUCED SLEEPING TIME IN RATS

Chloral hydrate 200 mg/kg i.v.		Chloral hydrate 100 mg/kg i.v.	
Control Sleep Times (min.)	Diphenoxylate-dosed[a] Sleep Times (min.)	Control Sleep Times (min.)	Diphenoxylate-dosed[a] Sleep Times (min.)
37	68	--[b]	24
37	78	--	31
40	63	--	31
44	70	--	31
38	45	--	16
49	49	--	36
38	59	--	31
42	55	--	42
	41	--	35
		--	44
Mean±SD 40.62±4.2	58.6±12.3		32.1±8.1

"t" 3.92

p>0.01

a 10 mg/kg diphenoxylate, p.o. 16-18 hours prior to dosing with flurazepam

b Animals did not lose righting reflex

TABLE 13

EFFECT OF DIPHENOXYLATE ON FLURAZEPAM-INDUCED SLEEPING TIMES IN RATS

Flurazepam 60 mg/kg i.v.		Flurazepam 30 mg/kg i.v.	
Control Sleep Time (min)	Diphenoxylate-dosed[a] Sleep Time (min)	Control Sleep Time (min)	Diphenoxylate-dosed[a] Sleep Time (min)
26	41	24	30
22	45	-[b]	27
21	46	6	18
25	39	-[b]	31
24	53	10	20
24	24	4	20
14	48	6	24
21	50	10	18
15	41	5	17
18	23	24	46
16	43	13	25
23	45	-[b]	56
22	45	15	53
22	54	-[b]	30
20	47	8	56
19		7	45
		-[b]	30
Mean ±S.D. 20.75±3.5	Mean ±S.D. 42.93±8.9	Mean ±S.D. 7.76±7.7	Mean ±S.D. 32.12±13.7
"t" 9.24		"t" 6.39	
p >0.01		p >0.01	

[a] 10 mg/kg p.o., 17-18 hr prior to dosing with flurazepam.

[b] Animal did not lose righting reflex.

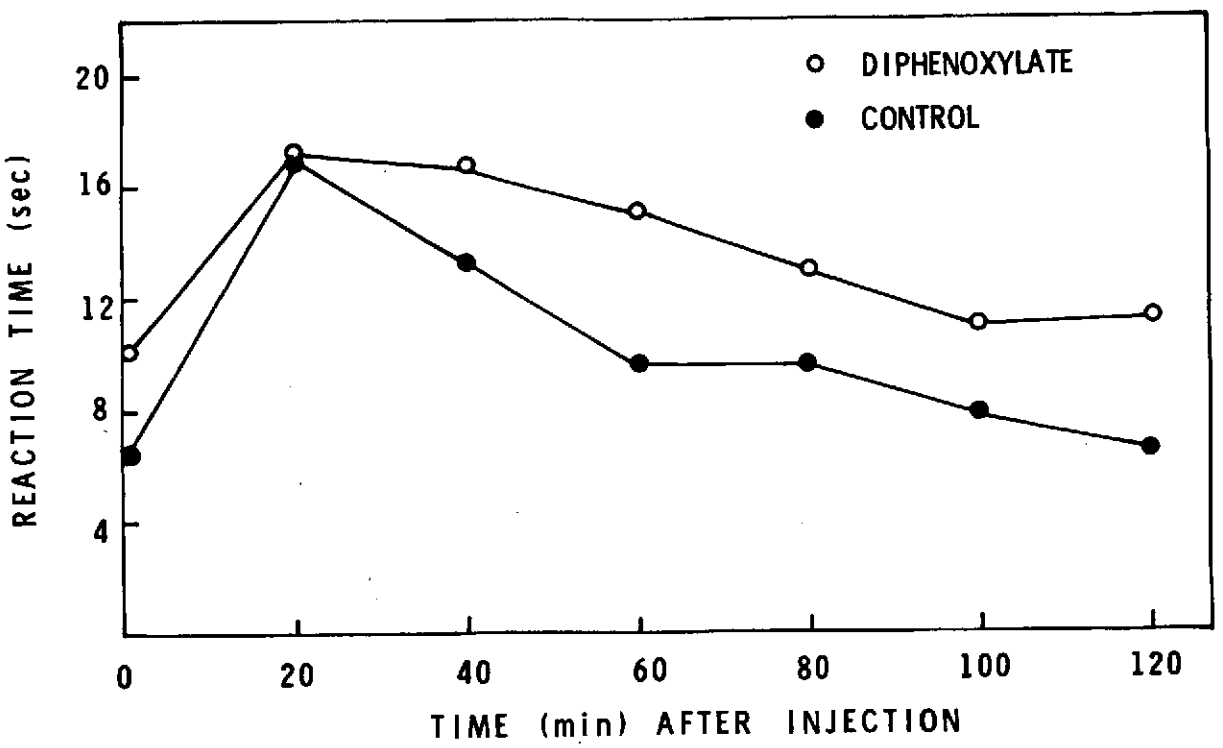


FIGURE 1

Meperidine-induced analgesic activity in control and diphenoxylate-pretreated rats - Experiment 1.

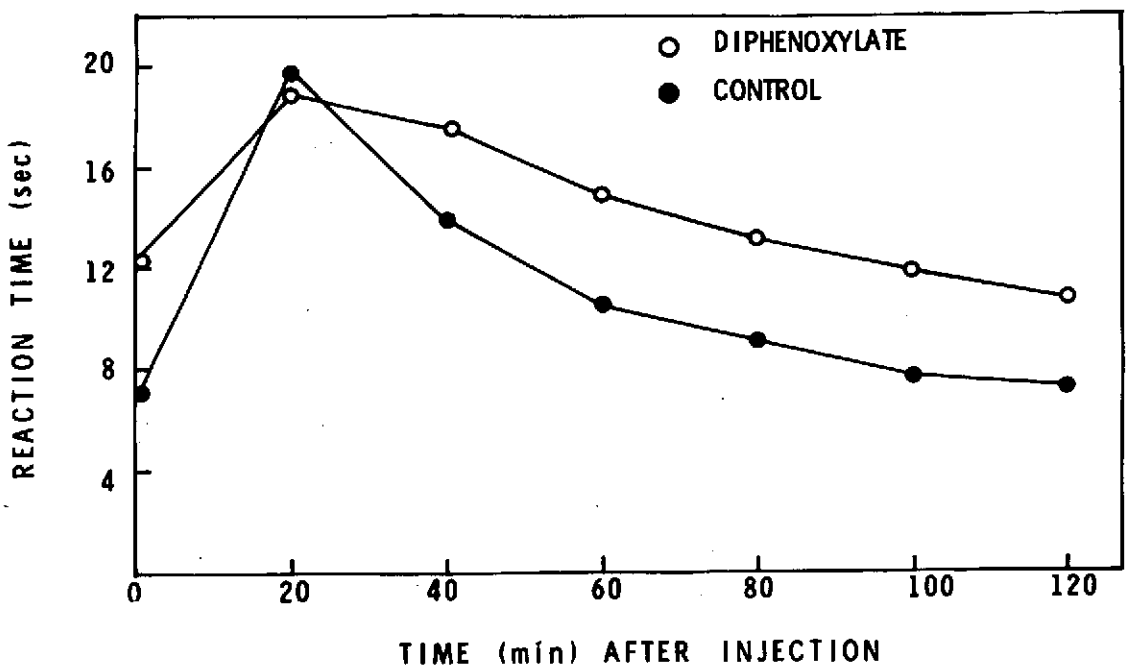


FIGURE 2

Meperidine-induced analgesic activity in control and diphenoxylate-pretreated rats - Experiment 2.

group in each experiment and the dose of meperidine was 30 mg/kg i.v. The percent analgesia was significantly higher in diphenoxylate-pretreated animals (57.5%) compared to control animals (33%)(Table 14).

TABLE 14

MEPERIDINE-INDUCED ANALGESIA IN CONTROL AND DIPHENOXYLATE-PRETREATED RATS

Time after Injection (min)	Mean Reaction Time (sec.) ±S.D. Control	Diphenoxylate-pretreatment
	EXPERIMENT 1	
0	6.4±2.6	10.5*±3.7
20	17.6±3.3	19.2 ±2.4
40	13.4±4.8	17.4*±3.6
60	9.8±4.4	15.6*±4.2
80	9.9±5.2	13.4*±4.4
100	8.1±3.7	11.5*±5.3
120	6.6±2.7	11.7*±5.6
Percent analgesia	33.0	56.5
	EXPERIMENT 2	
0	7.1±2.0	12.4*±4.9
20	19.8±0.5	19.0 ±2.3
40	14.0±4.7	17.7*±3.2
60	10.6±4.5	14.9*±4.7
80	9.2±3.5	13.3*±4.9
100	7.7±2.2	12.0*±4.7
120	7.4±1.9	10.8*±4.4
Percent analgesia	32.8	58.5

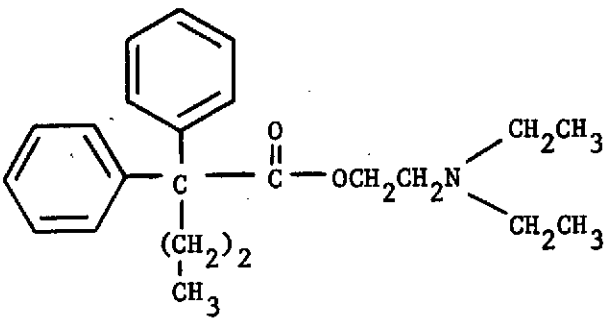
* Significantly greater than control ($p > 0.05$)

B. MECHANISM OF INTERACTION BETWEEN DIPHENOXYLATE AND SEDATIVE DRUGS.

Several possible mechanisms of action have been evaluated in order to explain the enhanced sedation in diphenoxylate-pretreated animals.

1. Drug Metabolism

The structural similarity of diphenoxylate to SKF-525A (III), a known potent inhibitor of hepatic drug metabolism, initially prompted the speculation that the potentiation of barbiturate action involved an inhibition of microsomal enzymes. However, studies reported in the final report for NASA contract NAS 9-9806 measuring microsomal hexobarbital oxidase in control and diphenoxylate-pretreated animals showed no difference in the two groups as defined by their K_m and V_{max} values.



SKF-525A III

2. Plasma and Excretion Pharmacokinetics of Sedative Drugs.

We evaluated the possibility that the enhanced sedation might relate closely to the pharmacokinetics of the sedative drugs in the diphenoxylate-pretreated rats. The barbiturates, pentobarbital, phenobarbital, hexobarbital, and secobarbital and chloral hydrate were chosen because they vary widely in their metabolism, excretion, and duration of

pharmacological activity.

Radiolabeled sedative drugs were administered i.v. to both control and diphenoxylate-pretreated rats. Drug equivalents in plasma, urine, and feces were determined. The plasma pharmacokinetics of total drug equivalents are presented in Tables 15-19 for all drugs, and illustrated for secobarbital and chloral hydrate in Figure 3. Statistically, the curves and data for all the barbiturates tested and for chloral hydrate are equivalent to those obtained from control animals. Radiolabeled flurazepam was not synthesized in time to be included in these studies; however, a subsequent study on a smaller scale leads one to the same conclusion.

Urinary excretion of drug equivalents was essentially the same for both groups of animals, as can be seen in Tables 20-24, with the possible exception of the chloral hydrate (Table 21). It appears that urinary excretion of chloral hydrate equivalents is increased in diphenoxylate-pretreated rats, although it must be pointed out that the standard deviation values are high. Feces were analyzed from the secobarbital, pentobarbital, and chloral hydrate experiments. The results are presented in Tables 25-27. Fecal output of drug equivalents in the diphenoxylate-pretreated rats is somewhat reduced and always delayed due to the action of the drug.

3. Pharmacokinetics of Secobarbital and Flurazepam Equivalents in Brain. In order to investigate further the pharmacodynamics of sedative drugs in diphenoxylate-pretreated animals, we compared the concentrations of secobarbital and flurazepam equivalents in blood and brain of control and diphenoxylate-pretreated animals. Four control and four diphenoxylate-pretreated rats were sacrificed at various time periods up to 2 hr after receiving either C^{14}-secobarbital or C^{14}-flurazepam. In addition, 6 control and 6 diphenoxylate-pretreated rats were

TABLE 15

CONCENTRATION OF SECOBARBITAL EQUIVALENTS IN PLASMA OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS[a]

Time	Control[b,c]	Diphenoxylate-treated[b,c]
1 min	39.32 ± 3.99	55.58 ± 9.69
2	38.38 ± 3.76	48.58 ± 5.60
5	37.40 ± 2.84	44.66 ± 6.01
10	35.85 ± 1.85	42.24 ± 4.78
15	33.04 ± 5.81	38.74 ± 4.26
30	36.04 ± 2.46	41.78 ± 3.83
45	34.70 ± 1.94	40.70 ± 5.69
60	31.36 ± 3.35	41.40 ± 6.23
75	29.15 ± 2.00	35.40 ± 5.37
90	26.55 ± 4.15	32.50 ± 5.58
2 hr	-[d]	27.90 ± 6.02
3	9.64 ± 2.62	13.94 ± 3.72
4	4.52 ± 1.47	-[d]
6	1.70 ± 0.66	3.28 ± 1.32
8	1.50 ± 0.45	2.50 ± 0.48
12	1.30 ± 0.12	1.52 ± 0.31
24	0.35 ± 0.10	0.42 ± 0.22

a Rats received either 10 mg/kg diphenoxylate or a comparable dose of 1% Tween 80/saline p.o. 17 hr prior to dosing with 30 mg/kg secobarbital i.v.

b Results expressed as mean µg/ml ±S.D. for 5 animals.

c The mean sleeping time ±S.D. for the control and diphenoxylate-treated animals were:

45.5±7.9 and 94.3±18.2, respectively.

d Sample lost.

TABLE 16

LEVEL OF CHLORAL HYDRATE EQUIVALENTS IN PLASMA OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS[a]

	Control[b,c] + Chloral Hydrate			Diphenoxylate[b,c] + Chloral Hydrate		
Rat No. / Time	1	2	Mean±S.D.	6	7	Mean±S.D.
1 min	189.78	227.14	208.5±26.4	234.83	275.48	255.2±28.7
2	212.88	203.46	208.2± 6.7	269.17	264.15	266.7± 3.5
5	249.77	240.82	245.3± 6.3	273.39	296.12	284.7±16.1
10	256.56	248.62	252.6± 5.6	291.30	317.97	304.6±18.8
15	247.90	270.15	259.0±15.7	282.66	257.43	270.1±17.8
30	259.90	298.10	278.9±27.0	299.94	280.77	290.4±13.6
45	233.49	291.12	262.3±40.7	284.91	285.55	285.2± 0.4
60	234.47	268.82	251.6±24.3	242.26	277.55	259.9±24.9
75	205.83	246.61	226.2±28.8	-[d]	-[d]	
90	185.84	175.34	180.6± 7.4	233.11	243.45	238.3± 7.3
2 hr	113.58	155.99	134.8±29.9	163.89	179.30	171.6±10.9
3	82.41	97.76	90.1±10.8	81.85	107.92	94.9±18.4
4	71.35	-[d]		77.72	89.86	83.8± 8.6
6	57.27	72.85	65.1±11.0	61.32	-[d]	
8	71.38	56.73	64.1±10.4	90.01	66.52	78.3±16.6
12	48.20	50.16	49.2± 1.4	72.89	47.93	60.4±17.6
24	-[d]	-[d]		-[d]	-[d]	
48	13.62	11.40	12.5± 1.6	12.07	14.31	13.2± 1.6

[a] Rat received either 10 mg/kg diphenoxylate or a comparable dose of 1% Tween 80/saline 17 hr prior to dosing with 200 mg/kg i.v. chloral hydrate.

[b] Results expressed as µg/ml.

[c] The mean sleeping time±S.D. for control and diphenoxylate-treated rats were: 35.2 ± 6.2 and 71.0 ± 3.7, respectively.

[d] Sample lost.

TABLE 17

CONCENTRATION OF PENTOBARBITAL EQUIVALENTS IN PLASMA OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS[a]

Time	Control[b,c]	Diphenoxylate-treated[b,c]
1 min	26.16 ± 6.44	25.12 ± 3.55
2	23.68 ± 4.94	21.51 ± 3.75
5	23.77 ± 3.25	21.64 ± 3.17
10	24.52 ± 5.71	21.32 ± 3.23
15	22.09 ± 0.85	21.20 ± 2.13
30	22.70 ± 2.13	21.68 ± 2.17
45	21.13 ± 1.25	20.10 ± 1.95
60	20.71 ± 1.65	19.13 ± 1.50
75	19.08 ± 2.12	18.37 ± 1.44
90	16.74 ± 1.75	15.82 ± 0.55
2 hr	12.86 ± 1.99	13.54 ± 1.19
3	7.33 ± 1.48	9.74 ± 2.00
4	3.92 ± 0.97	5.92 ± 1.54
6	1.13 ± 0.77	2.60 ± 0.65
8	0.96 ± 0.65	1.13 ± 0.67
12	0.50 ± 0.11	0.72 ± 0.60
24	0.30 ± 0.53	0.14 ± 0.11
48	0.04 ± 0.02	0.05 ± 0.02

[a] Rats received either 10 mg/kg p.o. diphenoxylate or a comparable dose of 1% Tween 80/saline 18 hr prior to dosing with 20 mg/kg pentobarbital i.v.

[b] Results expressed as mean µg/ml ±S.D. for 5 rats

[c] The mean sleeping time ±S.D. for control and diphenoxylate-treated rats were:

28.75 ± 6.09 and 66.43 ± 8.04

TABLE 18

CONCENTRATION OF PHENOBARBITAL EQUIVALENTS IN PLASMA OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS[a]

Time	Control[b,c]	Diphenoxylate-treated
1 min	276.89±67.72	296.50±44.99
2	252.38±75.99	258.52±20.18
5	239.82±24.49	221.42±16.36
10	234.24±21.96	226.00±42.49
15	235.36±25.94	216.72± 8.47
30	231.84±26.36	235.27±46.47
45	230.58±23.04	238.35±40.00
60	222.90±22.88	227.32±37.25
75	231.50±28.25	210.32±26.61
90	206.74±28.25	195.35±11.77
2 hr	211.82±38.87	189.15±18.30
3	214.96±53.29	168.95±11.28
4	187.76±23.45	161.50±11.79
6	170.30±36.78	142.62± 8.90
8	141.94±30.71	123.77±15.32
12	90.14±25.62	93.65± 9.93
24	32.78± 7.48	39.22± 5.37
48	2.10± 1.80	4.87± 1.97

a Rats received either 10 mg/kg p.o. diphenoxylate or a comparable dose of 1% Tween 80/saline 18 hr prior to dosing with 110 mg/kg phenobarbital i.v.

b Results expressed as mean µg/ml ±S.D.

c The mean sleeping time ± S.D. for control and diphenoxylate-treated rats were:

53.0±6.6 min and 96.8±3.7 min, respectively

TABLE 19

CONCENTRATION OF HEXOBARBITAL EQUIVALENTS IN PLASMA OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS[a]

Time	Control[b,c]	Diphenoxylate-treated[b,c]
1 min	101.06 ± 16.70	126.18 ± 19.38
2	106.22 ± 11.85	132.00 ± 14.47
5	125.68 ± 35.31	133.30 ± 10.57
10	129.00 ± 9.30	129.60 ± 12.81
15	129.44 ± 9.62	137.92 ± 10.98
30	135.96 ± 9.81	143.14 ± 9.67
45	137.14 ± 6.34	150.46 ± 7.58
60	134.86 ± 3.60	147.72 ± 6.57
75	126.20 ± 3.01	141.90 ± 2.41
90	117.00 ± 11.64	147.28 ± 9.13
2 hr	89.18 ± 3.21	116.60 ± 11.69
3	58.40 ± 21.32	75.56 ± 19.20
4	33.96 ± 12.13	51.72 ± 17.24
6	16.34 ± 4.79	24.54 ± 8.17
8	12.62 ± 1.26	17.12 ± 6.63
12	10.78 ± 1.16	11.54 ± 1.12
24	5.90 ± 0.32	6.30 ± 1.58

a Rats received either 10 mg/kg diphenoxylate or a comparable dose of 1% Tween 80/saline 17 hr prior to dosing with 80 mg/kg hexobarbital i.v.

b Results expressed as mean µg/ml ± S.D. for 5 animals.

c The mean sleeping time ±S.D. for the control and diphenoxylate-treated animals were:

28.4±3.6 and 45.8±6.7, respectively.

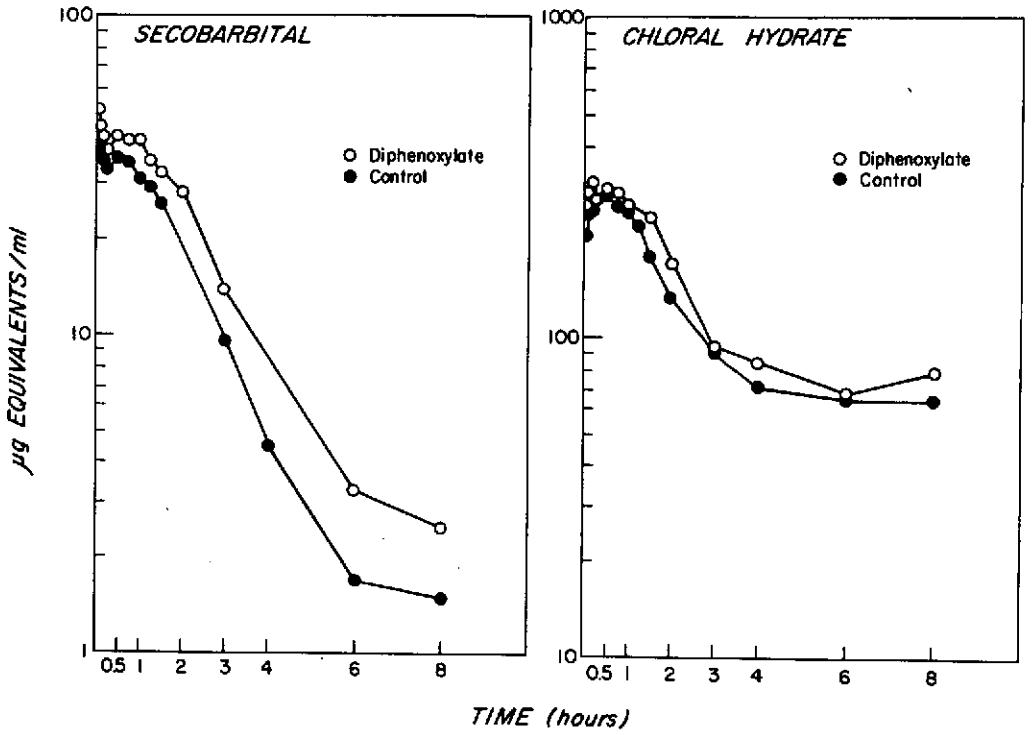


FIGURE 3

Effect of Diphenoxylate on Plasma Pharmacokinetics of Secobarbital and Chloral Hydrate Equivalents.

TABLE 20

CONCENTRATION OF SECOBARBITAL EQUIVALENTS IN URINE OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS

Time (hr)	Control (Drug equivalents - mg[a])						Mean ±S.D.	Diphenoxylate-treated (Drug equivalents - mg[a])						Mean ±S.D.
Rat No.	1	2	3	4	5	6		7	8	9	10	11	12	
0-6	0.18	2.06	1.47	1.88	2.42	1.60	1.60±0.77	1.87	2.27	0.85	1.67	2.39	2.05	1.85±0.56
6-9	0.39	0.45	0.21	0.25	0.10	2.19	0.60±0.79	0.36	0.01	0.81	0.23	0.77	0.25	0.41±0.32
9-12	0.12	0.13	0.05	0.11	0.10	0.13	0.11±0.03	0.13	0.01	0.08	0.13	0.07	0.16	0.49±0.37
12-24	0.21	0.10	0.16	0.02	0.14	0.11	0.25±0.37	0.22	1.21	0.29	0.35	0.58	0.30	0.49±0.37
24-48	0.94	0.03	0.05	0.02	0.40	0.04	0.25±0.37	0.05	0.05	0.12	0.10	0.50	0.04	0.14±0.18
Total mg excreted in 48 hr	1.83	2.79	1.94	2.28	3.16	4.07	2.68±0.85	2.64	3.56	2.16	2.47	4.31	2.81	2.99±0.80
% of Dose	50.1	74.5	49.8	61.3	83.0	106.0	70.8 ±22.2	73.6	78.1	53.8	64.8	103.2	79.4	75.3 ±16.9

[a] Results expressed total mg excreted per collection period.

TABLE 21

CONCENTRATION OF CHLORAL HYDRATE EQUIVALENTS IN URINE OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS

Time (hr)	Control (Drug Equivalents - mg)[a]							Diphenoxylate-treated (Drug Equivalents - mg)[a]					
Rat No.	1	2	3	4	5	6	Mean ±S.D.	7	8	9	10	11	Mean ±S.D.
0-3	1.35	14.80	6.17	0.83	8.81	4.10	6.01 ±5.2	7.39	11.10	8.40	16.94	13.83	9.7 ± 5.6
3-6	2.20	1.30	3.97	2.15	0.20	3.28	2.18 ±1.3	0.62	4.04	9.71	4.43	8.31	6.8 ± 4.8
6-9	0.60	0.90	0.67	0.89	0.25	0.07	0.56 ±0.33	9.24	0.83	0.60	0.73	1.71	2.4 ± 3.4
9-12	0.32	0.17	0.33	0.27	0.34	1.64	0.51 ±0.56	1.04	0.01	0.32	0.34	0.36	0.40± 0.34
12-24	1.25	0.27	1.64	0.32	0.65	0.71	0.81 ±0.54	0.97	0.83	0.45	0.69	0.77	0.68± 0.23
24-48	0.49	0.21	0.10	0.25	0.36	0.48	0.32 ±0.16	0.15	0.45	0.22	0.66	0.57	0.38± 0.21
Total mg excreted in 48 hr	6.21	17.65	12.88	4.71	10.61	10.28	10.4 ±4.7	19.41	17.26	19.75	23.79	25.55	20.5 ± 3.4
% of Dose	25	74	52	19	41	40	41.8 ±19.7	71	64	74	89	97	76.3 ±13.8

[a] Results expressed as total mg excreted per collection period.

TABLE 22

CONCENTRATION OF PENTOBARBITAL EQUIVALENTS IN URINE OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS

Time (hr)	Control (Drug Equivalents - mg[a])						Diphenoxylate-treated (Drug Equivalents - mg[a])					
Rat No.	1	2	3	4	5	Mean ±S.D.	6	7	8	9	10	Mean ±S.D.
0-6	1.53	1.79	0.90	1.52	1.19	1.39 ± 0.35	1.11	1.64	0.49	0.54	1.21	0.99 ± 0.48
6-9	0.11	0.10	0.17	0.16	0.10	0.13 ± 0.03	0.04	0.01	0.27	0.10	0.30	0.16 ± 0.16
9-12	0.03	0.09	0.08	0.04	0.16	0.08 ± 0.06	-[b]	0.16	0.20	0.11	0.16	0.16 ± 0.04
12-24	0.06	0.05	0.21	0.21	0.06	0.12 ± 0.08	0.35	0.11	0.30	0.27	0.19	0.24 ± 0.09
24-48	0.01	0.01	0.03	0.01	0.03	0.02 ± 0.01	0.04	0.06	0.09	0.05	0.02	0.05 ± 0.02
Total mg excreted in 48 hr	1.74	2.04	1.39	1.94	1.55	1.73 ± 0.27	1.55	1.98	1.35	1.07	1.98	1.59 ± 0.40
% of Dose	70.4	82.2	60.0	78.3	61.1	70.4 ± 9.9	57.0	72.3	44.7	44.3	72.3	58.1 ±13.9

a Results expressed as total mg excreted per collection period

b No urine excreted

TABLE 23

CONCENTRATION OF PHENOBARBITAL EQUIVALENTS IN URINE OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS

Time (hr)	Control (Drug Equivalents-mg[a])							Diphenoxylate-treated (Drug Equivalents-mg[a])						
Rat No.	1	2	3	4	5	6	Mean ±S.D.	7	8	9	10	11	12	Mean ±S.D.
0-6	5.49	1.62	2.33	2.55	3.92	2.00	2.98±1.45	2.10	1.10	0.0	0.0	0.71	0.0	0.65±0.84
6-9	0.02	0.25	0.65	0.47	1.38	0.17	0.49±0.48	0.12	0.06	2.26	4.22	0.03	1.98	1.44±1.69
9-12	0.66	0.34	1.15	0.88	0.48	0.52	0.67±0.29	0.60	1.03	0.23	1.58	0.87	0.89	0.87±0.45
12-24	3.35	1.85	2.35	2.58	2.00	2.64	2.46±0.53	2.12	2.38	5.10	3.29	3.63	3.39	3.32±1.06
24-48	1.61	0.92	1.05	1.10	0.81	1.63	1.19±0.35	0.16	2.44	2.11	1.79	0.46	2.43	1.56±1.00
Total mg excreted in 48 hr	11.03	4.98	7.53	7.58	8.59	6.96	7.73±1.99	5.10	7.01	9.70	10.88	5.70	8.69	7.85±2.28
% of Dose	75.5	42.2	59.2	62.6	64.1	52.7	59.7±11.2	37.1	48.7	59.9	75.0	39.3	59.9	53.3±14.4

[a] Results expressed as total mg excreted per collection period

TABLE 24

CONCENTRATION OF HEXOBARBITAL EQUIVALENTS IN URINE OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS

Time (hr)	Control (Drug equivalents - mg[a])							Diphenoxylate-treated (Drug equivalents - mg[a])				
Rat No.	1	2	3	4	5	6	Mean ±S.D.	7	8	9	10	Mean ±S.D.
0-6	9.44	8.64	10.62	11.21	9.90	7.80	9.60±1.26	6.33	7.60	8.31	8.62	7.72±1.02
6-9	2.15	1.36	0.99	0.82	1.85	0.47	1.27±0.64	3.34	-[b]	3.02	1.10	2.49±1.21
9-12	0.14	0.57	0.56	0.33	1.10	2.35	0.84±0.81	2.32	-[b]	1.10	1.42	1.61±0.63
12-24	1.87	0.71	0.72	0.32	1.04	1.17	0.97±0.53	1.05	7.80	1.90	1.47	1.47±0.43
24-48	0.26	0.28	0.20	0.13	0.24	0.35	0.97±0.53	0.52	0.21	0.11	0.17	1.47±0.43
Total mg excreted in 48 hr	13.86	11.56	13.09	12.79	14.14	12.14	12.93±0.99	13.56	15.61	14.44	12.78	14.10±1.22
% of Dose	122.3	117.8	123.5	130.5	125.3	114.6	123.8 ±4.4	120.9	137.1	125.7	118.2	126 ±9.2

[a] Results expressed as total mg excreted per collection period

[b] No urine excreted

TABLE 25

CONCENTRATION OF SECOBARBITAL EQUIVALENTS IN FECES OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS

Time (hr)	Control (Drug Equivalents - mg[a])							Diphenoxylate-treated (Drug Equivalents - mg[a])						
Rat No.	1	2	3	4	5	6	Mean ±S.D.	7	8	9	10	11	12	Mean ±S.D
0-6	0.24	0.26	0.01	0.03	0.01	-	0.11±0.13	0.01	0.08	0.01	0.001	0.01	0.01	0.02±0.02
6-9	0.29	0.33	0.44	0.26	0.66	0.37	0.39±0.14	0.03	0.15	0.01	0.03	0.03	0.01	0.04±0.05
9-12	0.11	0.20	0.19	0.21	0.21	0.32	0.21±0.07	0.33	0.09	0.002	0.48	0.03	0.03	0.16±0.19
12-24	0.18	0.10	0.16	0.17	0.11	0.19	0.15±0.04	0.23	0.32	0.18	0.13	0.33	0.22	0.23±0.08
24-48	0.05	0.01	0.04	0.03	0.07	0.04	0.04±0.02	0.07	0.07	0.16	0.12	0.05	0.06	0.09±0.04
Total mg excreted in 48 hr	0.87	0.90	0.84	0.70	1.06	0.92	0.88±0.12	0.67	0.71	0.36	0.76	0.45	0.33	0.55±0.19
% of Dose	24	24	22	19	28	24	23.5 ±2.9	18	16	9	20	11	10	14.0 ±4.6

[a] Results expressed as total mg per collection period.

TABLE 26

CONCENTRATION OF CHLORAL HYDRATE EQUIVALENTS IN FECES OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS

Time (hr)	Control (Drug Equivalents - mg[a])							Diphenoxylate-treated (Drug Equivalents - mg[a])					
Rat No.	1	2	3	4	5	6	Mean ±S.D.	7	8	9	10	11	Mean ± S.D.
0-3	0.04	0.002	-[b]	0.02	-[b]	0.003	0.02±0.02	-[b]	0.002	0.004	0.002	0.005	0.003±.001
3-6	0.05	0.02	0.07	0.03	0.003	0.004	0.03±0.03	0.07	0.02	0.02	0.02	0.008	0.03 ±.02
6-9	0.01	0.16	0.03	0.02	0.02	0.06	0.05±0.06	0.02	0.04	0.03	0.007	0.02	0.02 ±.01
9-12	0.02	0.002	0.02	0.03	0.04	0.04	0.03±0.01	0.09	0.03	0.01	0.04	0.006	0.03 ±.03
12-24	0.07	0.06	0.05	0.07	0.06	0.05	0.06±0.001	0.08	0.07	0.09	0.08	0.05	0.07 ±.01
24-48	0.05	0.06	0.04	0.12	0.07	0.02	0.06±0.03	0.08	0.09	0.12	0.05	0.06	0.08 ±.03
Total mg excreted in 48 hr	0.24	.30	0.21	0.28	0.19	0.17	0.23±0.05	0.34	0.25	0.27	0.19	0.15	0.24 ±.07
% of Dose	1.0	1.3	0.9	1.2	0.8	0.7	0.98±0.23	1.3	1.0	1.0	0.7	0.6	0.92 ±.3

a Results expressed as mg per collection period.

b No feces collected

TABLE 27

CONCENTRATION OF PENTOBARBITAL EQUIVALENTS IN FECES OF CONTROL AND DIPHENOXYLATE-PRETREATED RATS

Time (hr)	Control (Drug Equivalents - mg[a])						Diphenoxylate-treated (Drug Equivalents - mg[a])					
Rat No.	1	2	3	4	5	Mean ±S.D.	6	7	8	9	10	Means ±S.D.
0-6	0.179	0.004	-[b]	0.0003	-[b]	0.037±0.080	0.003	0.004	0.002	0.004	0.004	0.003±0.001
6-9	0.175	0.234	0.195	0.024	0.188	0.163±0.081	0.002	0.005	0.001	0.001	0.009	0.004±0.003
9-12	0.061	0.085	0.111	0.195	0.179	0.126±0.059	0.004	0.078	-[b]	0.006	0.026	0.023±0.003
12-24	0.064	0.081	0.108	0.130	0.088	0.094±0.026	0.099	0.227	0.162	0.140	0.123	0.150±0.049
24-48	0.009	0.012	0.094	0.044	0.034	0.039±0.034	0.036	0.031	0.024	0.046	0.025	0.032±0.009
Total mg excreted in 48°	0.488	0.416	0.508	0.444	0.489	0.469±0.038	0.144	0.345	0.189	0.197	0.187	0.212±0.077
% Dose	19.6	16.8	20.9	17.5	19.3	18.8 ±1.7	5.3	12.6	6.2	8.2	6.8	7.8 ±2.9

[a] Results expressed as total mg per collection period

[b] No feces excreted

sacrificed immediately upon waking. Figures 4 and 5 show that the concentration of drug equivalents in the blood and the brain is essentially the same for both groups of animals. In the secobarbital experiment, the control animals slept for an average of 58 min, while the diphenoxylate-pretreated rats averaged 109 min. Although the sleeping times are approximately double in diphenoxylate animals, the concentration of secobarbital equivalents at waking is less in both the brain and blood of the diphenoxylate-treated animals compared to control animals. The same is true for the level of flurazepam equivalents in control animals which slept for 17 min and treated rats which slept for 55 min. One would have expected equivalent concentrations of sedative drug equivalents if the sedative drug itself had mediated this increased sleeping time.

4. Effect of Dose and Time of Administration of Diphenoxylate on Sedative-Induced Sleeping Times.

Janssen and associates in Belgium have shown that diphenoxylate and its metabolites distribute into the brain of rats and remain there for long periods of time (Table 28). Thus, we evaluated the effect of varying the dose and the time of administration of diphenoxylate in combination with secobarbital and chloral hydrate. Rats were dosed orally with diphenoxylate at four concentrations, and the sedative drugs were administered at four time periods thereafter (Tables 30-33). As the concentration of diphenoxylate increased, the length of sleeping times with secobarbital increased significantly (Table 29). When secobarbital was administered 1 hr after the animals received 0.16 mg/kg diphenoxylate, there was no significant increase in sleeping times. However, when the secobarbital was administered 40 hr following the 0.16 mg/kg dose of diphenoxylate, sleeping times were increased. A similar increase can be seen with the 0.625 and 2.5 mg/kg doses of diphenoxylate. Thus, as the length of time between dosing with diphenoxylate and dosing with secobarbital increased, the length of sleeping times are significantly increased. This is not the case at the 10 mg/kg concentration of diphenoxylate, as the increase in sleeping times of treated animals

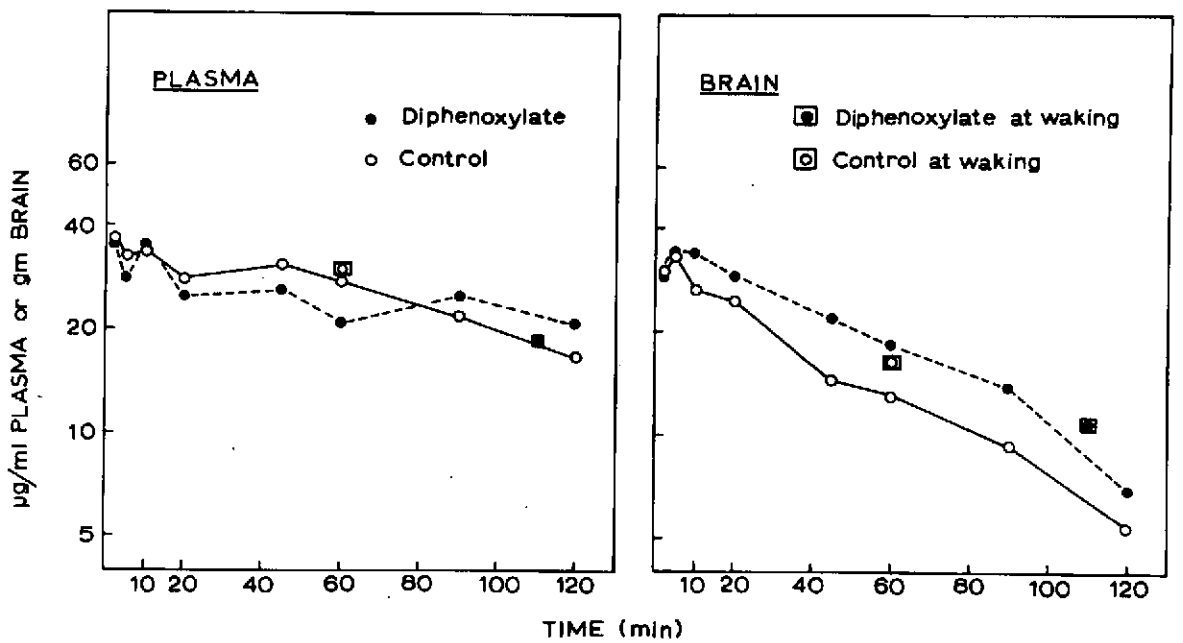


FIGURE 4

Effect of diphenoxylate on the pharmacokinetics of secobarbital equivalents in plasma and brain.

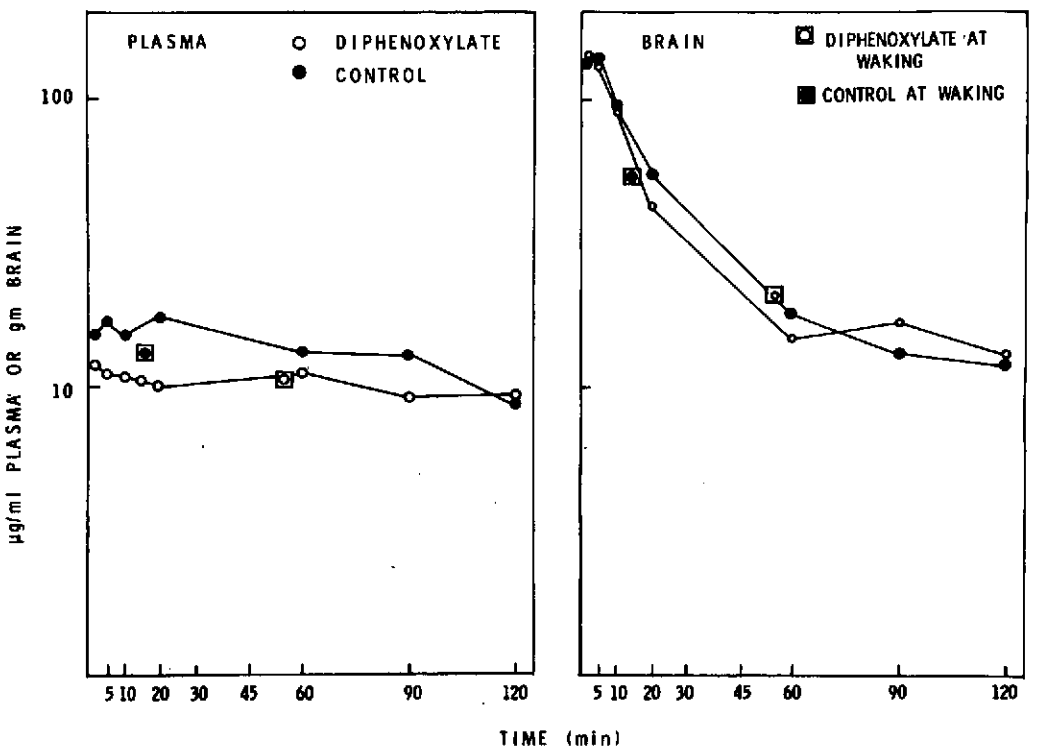


FIGURE 5

Effect of diphenoxylate on the pharmacokinetics of flurazepam equivalents in plasma and brain.

TABLE 28

CONCENTRATION OF DIPHENOXYLATE (R1132) AND DIFENOXINE (R15403) IN BRAIN AND BLOOD OF RATS*

Dose of Diphenoxylate (mg/kg)	0.16			0.63			2.5			10		
Time (Hr)	Total H^3 Equiv.	R1132	R15403	Total H^3 Equiv.	R1132	R15403	Total H^3 Equiv.	R1132	R15403	Total H^3 Equiv.	R1132	R15403
					Brain (nanograms)							
1	17	5		41	13		99	31		241	76	
2	16	3	4	41	7	9	106	19	21	270	50	48
16	11	0	2	36	2	8	114	6	27	357	20	91
32	9	0	1	32	1	7	110	5	27	383	19	102
64	6	0	1	25	1	5	101	4	22	407	18	93
					Blood (nanograms)							
1	1167	459		2633	1036		5970	2349		13597	5351	
2	684	177	208	1667	433	459	4086	1061	1016	10066	2615	2259
16	156	5	121	494	17	374	1577	54	1160	5061	174	3621
32	92	1	50	326	6	203	1155	24	826	4117	87	3390
64	56	0	11	223	2	64	887	11	372	3551	44	2177

* J.J.P. Heykants, P.J. Lewi, and P.A.J. Janssen. Difenoxine (R15403), The Active Metabolite of Diphenoxylate (R1132). Part 4: Distribution in the Rat of Diphenoxylate and Difenoxine. Arzneim-Forsch (Drug Res.) 22:520, 1972.

TABLE 29

SUMMARY: TIME AND DOSE EFFECTS OF DIPHENOXYLATE ON DRUG-INDUCED SLEEPING TIMES

DIPHENOXYLATE (mg/kg)	0.16	0.625	2.5	10
	PERCENT OF CONTROL SLEEPING TIME			
PRETREATMENT (HOURS)	SECOBARBITAL			
1	116	133*	156*	203*
16		149*	177*	209*
40	133*	157*		210*
	CHLORAL HYDRATE			
1	105	113		191*
16				171*
40	107	92		131*

* Significantly greater than control. Based on original data.

TABLE 30

EFFECT OF DIPHENOXYLATE[a] ON SECOBARBITAL-INDUCED[b] SLEEPING TIMES IN RATS - EXPERIMENT 1

Time of dosing with diphenoxylate prior to receiving secobarbital	40 hr	8 hr	1 hr	Control[c]
	57	65	55	23
	60	61	60	24
	56	51	63	37
	68	56	40	36
	71	66	55	25
	54	62	60	32
	67	66	66	28
	63	58	59	29
	51	66		26
	62	68		31
Mean ±S.D.	60.9 ± 6.5	61.9 ± 5.4	57.3 ± 7.9	29.1 ± 4.9
"t" value plus probability factor	t = 12.354 p<0.01	t = 14.225 p<0.01	t = 9.306 p<0.01	

a Dose of diphenoxylate was 10 mg/kg p.o.

b Dose of secobarbital was 30 mg/kg i.v.

c Control animals received secobarbital only.

TABLE 31

EFFECT OF DIPHENOXYLATE ON SECOBARBITAL-INDUCED[a] SLEEPING TIMES IN RATS - EXPERIMENT 2

Time	1 hr prior to receiving secobarbital				16-18 hr prior to receiving secobarbital			
Dose of Diphenoxylate (p.o.)	10 mg/kg	2.5 mg/kg	0.625 mg/kg	Controls[b]	10 mg/kg	2.5 mg/kg	0.625 mg/kg	Controls[b]
	86	75	64	43	98	100	71	60
	87	63	64	44	122	121	64	64
	97	77	46	40	95	95	92	57
	93	49	67	43	105	98	70	53
	88	66	64	37	102	110	61	43
	91	58	64	37	102	95	97	54
	89	75	64	41	92	87	109	50
	86	67	42	44	89	87	98	52
	88	67	40	48	87	107	92	54
		70		51	91	69	56	56
Mean ±S.D.	89.4±3.6	66.7±8.5	57.2±11.1	42.8±4.4	98.3±10.3	96.9±14.3	81.0±18.6	54.3±5.6
"t" value plus probability factor	t=25.084 p<0.01	t=7.896 p<0.01	t=3.794 p<0.01		t=11.868 p<0.01	t=8.772 p<0.01	t=4.347 p<0.01	

[a] Dose of secobarbital was 30 mg/kg i.v.

[b] Control animals received 1% Tween 80/saline (diphenoxylate vehicle) at 0.5 ml/100 g rat either 1 hr or 16-18 hr prior to receiving secobarbital.

TABLE 32

EFFECT OF DIPHENOXYLATE ON SECOBARBITAL-INDUCED[a] SLEEPING TIMES IN RATS - EXPERIMENT 3

Time prior to receiving Secobarbital	40 hr			8 hr	1 hr			
Dose of Diphenoxylate (p.o.)	2.5 mg/kg	0.625 mg/kg	0.16 mg/kg	0.16 mg/kg	2.5 mg/kg	0.625 mg/kg	0.16 mg/kg	Control[b]
	52	67	53	42	57	52	51	41
	50	79	47	39	61	58	50	35
	51	67	57	50	60	41	37	37
	46	61	53	35	56	52	46	34
	50	61	41	48	66	57	36	35
	43	57	60	34	55	46	48	41
	60	55	56	34	60	49	54	45
	43	57	56	43	70	36	49	39
	52	56	43	43	70	60	32	36
	47	53		35		56	50	43
Mean ±S.D.	49.4±5.0	61.3±7.8	51.8±6.6	40.3±5.8	61.6±5.7	50.7±7.8	45.3±7.5	39.1±4.4
"t" value plus probability factor	t=4.890 p<0.01	t=7.839 p<0.01	t=4.985 p<0.01	t=0.521 p<0.7	t=9.690 p<0.01	t=4.096 p<0.01	t=2.255 p<0.02	

[a] Dose of secobarbital was 30 mg/kg i.v.

[b] Control animals received 1% Tween 80/saline (diphenoxylate vehicle) at 0.5 ml/100 g rat 1 hr prior to receiving secobarbital.

TABLE 33

EFFECT OF DIPHENOXYLATE ON CHLORAL HYDRATE[a]-INDUCED SLEEPING TIMES IN RATS

Time prior to receiving Chloral Hydrate	40 hr			8 hr	1 hr			
Dose of Diphenoxylate (p.o.)	10 mg/kg	0.625 mg/kg	0.16 mg/kg	0.16 mg/kg	10 mg/kg	0.625 mg/kg	0.16 mg/kg	Control[b]
	55	37	44	39	78	43	30	31
	38	34	30	39	71	49	37	35
	53	36	47	40	60	32	40	42
	43	40	46	31	73	45	40	40
	50	40	40	37	63	45	39	43
	39	30	44	37	76	29	40	36
	54	42	41	34	76	59	35	39
	51	33	30	40	75	29	40	31
		40	44	35	56		45	34
		23	36		70			35
Mean ±S.D.	47.8±6.8	33.5±7.3	40.2±3.1	36.9±3.1	69.8±7.6	41.4±10.6	38.4±4.2	36.6±4.2
"t" value plus probability factor	t=4.300 p<0.01	t=1.164 p<0.2	t=1.520 p=0.2	t=0.175 p<0.9	t=12.091 p<0.01	t=1.317 p<0.3	t=0.933 p<0.4	

[a] Dose of chloral hydrate was 200 mg/kg i.v.

[b] Control animals received 1% Tween 80/saline (diphenoxylate vehicle) at 0.5 ml/100 g rat 8 hr prior to receiving chloral hydrate.

over control animals appears to peak at approximately 200%.

With chloral hydrate as the sedative drug, the only significant increase in sleeping times occurred with the highest dose of diphenoxylate. Also, as the time between dosing with diphenoxylate and dosing with this high dose of chloral hydrate increased, the sleeping times, though still significant when compared with controls,decreased (Table 29). This may relate to the reported decrease in the parent drug in the brain during the pretreatment period. In addition, the absence of any enhanced sleeping time at the lower doses of diphenoxylate with chloral hydrate may be explained by the low concentration of diphenoxylate and its short duration in the brain.

Taking into account Janssen's observations concerning the pharmacokinetics of diphenoxylate, which show that the concentration of diphenoxylate decreases with time but the concentration of metabolites of diphenoxylate increases with time, one could suggest that the interaction between diphenoxylate and secobarbital may be associated with metabolites of diphenoxylate, whereas with chloral hydrate, it may be the diphenoxylate *per se*. The degree of depression, or action of the sedative drugs, depends not only on the particular drug, the dose, and the rate of administration, but also on the degree of excitability of the CNS at the time of administration and the extent to which previous experience with drugs has induced tolerance (15).

V. CONCLUSION

The increase in pharmacological activity when sedative drugs are used in combination with diphenoxylate cannot be explained on the basis of inhibition of drug metabolism or pharmacokinetic differences of the sedative drugs in diphenoxylate-pretreated animals. More than likely, it is the diphenoxylate *per se* and/or metabolites that remain in the brain for such long periods of time that when sedative drugs are administered, an additive effect can be seen as manifested in increased sleeping times or a higher level of meperidine analgesia.

Diphenoxylate is the drug of choice in many instances for the control of diarrhea. Since the work reported in this contract has shown that diphenoxylate can affect the pharmacodynamics of three classes of drugs - sedative drugs, analgesics, and urinary antiseptics - caution should be advised in administering drugs, especially CNS-acting drugs, to diphenoxylate-treated subjects.

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